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Chromatin immunoprecipitation-deep sequencing (ChIP-seq) and ChIP-qPCR

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An abbreviated version of this protocol was published in eLIFE in Jun 2018

H3.3K27M mutant proteins reprogram epigenome by sequestering the PRC2 complex to poised enhancers

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Detailed protocol

ChIP-Seq and qPCR protocol

A. Fixation and Chromatin shearing.

1. Grow cells in 10 cm plates to around 80-90% confluency
2. Take out plates from the incubator and incubate at RT for 10 minutes.
3. Add 0.27 ml of freshly prepared 37% PFA to a plate (10 ml medium) and incubate for 10 minutes at RT after swirling. Or, use 0.625 ml of the commercial 16% w/v formaldehyde solution (Thermo Scientific Cat. #: 28906) to fix cells.
4. Add 0.5 ml of 2.5 M Glycine, swirl the plate, and incubate at RT for 5 minutes.
5. Wash cells with 1X cold TBS twice. And remove completely any residual TBS.
6. Add 3 - 5 ml of cell lysis buffer (10mM Tris HCl, pH7.5, 10 mM NaCl, 0.5% NP-40)to a plate depending on cell number in the plate. Around 4X10^6 cells per 1 ml of lysis buffer.
7. Harvest cells by scraping and collect lysates into a 15 ml tube or 1.5 ml microcentrifuge tube.
8. Vortex and incubate on ice for 10 minutes.
9. Centrifuge (3,000 rpm x 5 min, 4 °C: table top) and remove supernatant.
10. Add the 1/2 volume (cell lysis buffer) of the MNase digestion buffer (20 mM Tris-HCl, pH7.5, 15 mM NaCl, 60 mM KCl, 1 mM $CaCl_2$)and briefly vortex.
11. Centrifuge (3,000 rpm x 5 min, 4 °C: table top) and add the MNase digestion buffer (added Protease inhibitor cocktail). **For 3 -4 X 10^6cells, 0.5 ml of the MNase digestion buffer is used.**
12. Mix very well by pipetting and transfer into a 1.5 ml microcentrifuge tube. **Reaction setup: 3 – 4 X 10^6cells /500 ul reaction volume + 5 ul of diluted enzyme**
13. Dilute MNase (NEB, M0247S, 2000 gel units/ul)to 1:10 with the MNase digestion buffer.
14. Add 5 µl of diluted MNase to each tube and incubate in the Isotemp (37 °C, 1000 rpm x 20 minutes). In this condition, at least 80 – 90 % of chromatins are digested into mono- and di-nucleosomes.
15. Add 500 µl of of 2X Stop/ChIP buffer I (100 mM Tris-HCl, pH8.1, 20 mM EDTA, 200 mM NaCl, 2% Triton X-100, 0.2% Sodium deoxycholate)to samples and mix by brief vortexing.
16. Sonicate the samples in the Diagenode Bioruptor (10 cycles of 30"-on, 30"-off). The exact number of cycles should be tested and adjusted around half a year or using different Diagenode Bioruptor. When run gel by DNA gel, the sonicated DNA should be mainly at mono-nucleosome size. Do not over-sonicate to lower than the mono-nucleosome size.
17. Centrifuge (15,000 rpm x 10 minutes, at 4°C) and transfer the supernatant to a new tube. Save samples for analysis of input chromatins (Measure the concentration of chromatin DNA by Qubit).
18. Save 1% of input. In case 950 ul of supernatant is used for ChIP, then take (9.5 µl of supernatant + 9.5 µl of 2X elution buffer + 91 µl of 1X elution buffer) and save it at -20 °C.

For the normalization of ChIP efficiency, the chromatin prepared similarly from S2 or Sf9 cells was added to reach to 1 to 5% of total chromatin. (Use same percentage in all the experimental samples).

B. Chromatin immunoprecipitation.

19. Add IgG or antibody of your interest (2- 10 µg) to the supernatant and rock at 4°C overnight. In case the primary antibody is mouse IgG, we add 2 µg of Bridging Antibody for Mouse IgG (Active motif 53017) and rock at 4°C for 2 hours.
20. Add 30 µl of pre-washed protein G-agarose beads ((Protein G Mag Sepharose® Xtra, washed twice with 1 ml 1x ChIP buffer) to each sample. Rock at 4 °C for 3 hours.
21. Centrifuge at 9000 rpm (microcentrifuge) and remove supernatant.
22. Wash beads at 4°C; 1ml of 1 x ChIP buffer, 1ml of 1 x ChIP buffer (5 min wash on rocker), 1ml of High salt buffer (ChIP buffer + 0.5 M NaCl), 1ml of High salt buffer (ChIP buffer + 0.5 M NaCl) (5 min wash on rocker), 1 ml of Tris/LiCl buffer, 1 ml of Tris/LiCl buffer (5 min wash on racker), 1 ml of TE buffer twice.
23. Add 50 ul of 1 X elution buffer to beads and incubate at 65 °C for 15 min (vortex 5 sec for every 3 min). Repeat elution with 50 ul of 1x elution buffer and combine eluents.
24. Cross-link reversal at 65 °C overnight. At this step, process the saved 1% input.

25. Add 2 µl of DNase-free RNase A (10 mg/ml, Thermo Scientific Cat. #: EN0531) and mix it, and incubate at 37 °C for 1 hour.
26. Add 10 µl of Proteinase K (20 mg/ml, Ambion, Cat. #: AM2546) and mix it, and incubate at 37 C for 2 hours.

C. Purification of DNA and qPCR.

27. Add 500 µl of buffer PB (from the Qiagen PCR purification kit) and mix very well.
28. Load onto the Qiagen MinElute column and centrifugation (12,000x g, 1 min, RT).
29. Discard the passthrough and add 650 µl of wash buffer (from the Qiagen PCR purification kit) on to the column. Centrifugation (12,000x g, 1 min, RT) and discard the passthrough.
30. Centrifugation at maximum speed for 1 minute.
31. Add 13 µl of elution buffer (from the Qiagen PCR purification kit) and incubate for 1 minute.
32. Transfer the column to clean microcentrifuge tube and centrifugation at maximum speed for 1 minute.
33. For evaluation of ChIP

- Measure DNA concentration by the Qubit assay: use 1 µl of ChIP product
-Check the enrichment by realtime PCR: dilute 1 µl of ChIP product 5 or 10 fold. 1 or 2µl of diluted ChIP product will be used for real-time PCR analysis.
-In case DNA is more than 1 ng with antibody of your interest and ChIP enrichment is good, then prepare NGS sequencing library.

D. Preparation of sequencing library.

34. 10 ng ChIP and input DNA were processed for library preparation by following the Ovation ultralow DR Multiplex kit (NuGEN). If ChIP DNA is less than 10 ng, use all of ChIP product.

Cell lysis buffer (extraction buffer)

10mM Tris HCl, pH7.5
10 mM NaCl
0.5% NP-40

MNase digestion buffer

20 mM Tris-HCl, pH7.5
15 mM NaCl
60 mM KCl
1 mM $CaCl_2$

2X Stop/ChIP buffer I

100 mM Tris-HCl, pH8.1,
20 mM EDTA
200 mM NaCl
2% Triton X-100
0.2% Sodium deoxycholate

High salt buffer (1X Stop/ChIP buffer + 0.5 M NaCl)

1 ml of 1X Stop/ChIP buffer + 72 ul of 5M NaCl

Tris/LiCl buffer

10 mM Tris-HCl, pH8.0,
0.25 M $LiCl_2$
0.5% NP-40
0.5% Sodium deoxycholate
1 mM EDTA

2X TE buffer : dilute to 1 X to use

100 mM Tris-HCl, pH8.0
20 mM EDTA

Elution buffer (1 X)

10 mM Tris-HCl, pH8.0
10 mM EDTA
150 mM NaCl
5 mM DTT
1% SDS

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fang, D. and Zhang, Z. (2020). Chromatin immunoprecipitation-deep sequencing (ChIP-seq) and ChIP-qPCR. Bio-protocol Preprint. bio-protocol.org/prep412.
2. Fang, D., Gan, H., Cheng, L., Lee, J., Zhou, H., Sarkaria, J. N., Daniels, D. J. and Zhang, Z.(2018). H3.3K27M mutant proteins reprogram epigenome by sequestering the PRC2 complex to poised enhancers. eLIFE. DOI: 10.7554/eLife.36696